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SIMULANTS FOR TRANSITION STATES

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Catalytic antibodies are proteins that catalyze chemical reactions by binding to the transition state and lowering the energy of activation. Transition state analogs are stable compounds that mimic the geometry and electronic structure of the transition structure. These haptens are conjugated to proteins and used to elicit the desired immunoglobulins. Catalytic antibodies have been used to accelerate hydrolysis and ammonolysis reactions, *cis-trans* isomerizations, Diels-Alder reactions, and to alter the product ratios for cyclization reactions.

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PREFACE

The work described in this report was authorized under Project No. 10262622A553, CB Defense/General Investigation. This work began in November 1994 and was completed in February 1995. The results described in the report were presented at the 9th International Simulant Workshop held at Edgewood, MD, on 7-8 March 1995.

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SIMULANTS FOR TRANSITION STATES

INTRODUCTION

Simulants are selected because some of their chemical and physical properties are similar to those of the compound or material simulated. The critical property or properties to be simulated vary depending on the particular use or application. In most cases, a stable compound is selected as a simulant for another stable compound. This report will address another aspect of simulation - the use of a stable compound to simulate the geometry and electronic properties of a high energy transition state that has no finite lifetime. The principal application for simulants for transition structures is in the development of catalytic antibodies.

During a chemical reaction, the reactants move from an energy minimum up a potential energy surface to an energy maximum, commonly called the transition state or transition structure, and then become the reaction products as they move down the surface to another energy minimum. The energy required to reach the energy maximum is the energy of activation. Reactions with small energies of activation proceed quickly while those requiring more energy are slower. Catalytic antibodies are proteins designed to bind to and thereby stabilize the transition structure.[1] The binding interactions lower the energy of activation and thereby enhance the reaction rate. If two or more species react to form a single transition structure, the catalysis may accelerate the reaction by helping to overcome unfavorable entropy effects. The binding of the protein to the transition structure occurs through electronic, hydrogen bonding, hydrophobic, and other interactions.

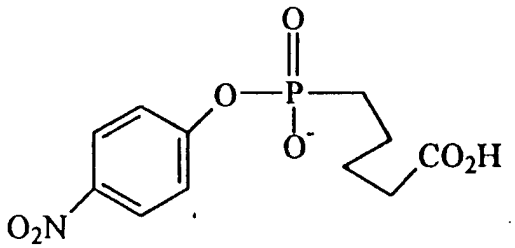
One of the first examples of successful transition state simulation was the use of phosphate compounds to mimic the geometry of carboxylic ester hydrolysis. During hydrolysis, the planar sp^2 carbon atom is converted to a tetrahedral structure upon addition of hydroxide. Phosphates are ideal simulates for this transition structure because their polar bonds, tetrahedral geometry, and O alkyl components are very similar to actual transition structures. Figure 1 illustrates the use of antibodies to catalyze the hydrolysis of a carbonate ester.[2] Antibodies were produced against the transition state analog, p-nitrophenyl-phosphorylcholine. The resulting antibodies have a binding constant of 1.4×10^6 with the analogous carbonate. In the pH range from 6.0 to 8.0, the rate of acceleration for hydrolysis was about 700 per molecule of antibody.

Unlike the more reactive esters, the hydrolysis of amide bonds occurs relatively slowly at room temperature even at elevated pH's. The transition state is a zwitterionic tetrahedral species that is reached via a series of rate limiting proton transfers. An effective catalytic antibody must possess bifunctional properties that permit it to stabilize the oxyanion and also protonate the amide nitrogen for easy expulsion. Figure 2 illustrates the phosphonoamidate that was used to generate the antibodies.[3] Hydrolysis of the corresponding substrate proceeded via Michaelis-Menton kinetics with a rate acceleration of 250,000.

In addition to accelerating hydrolysis reactions, antibodies have been used to catalyze the formation of new bonds. The cyclic phosphonate depicted in Figure 3 was used to produce antibodies that catalyzed the formation of the N-aryl amide by addition of diamino benzene to the cyclic lactone, 6-(acetamidomethyl)valerolactone.[4] The reaction proceeds by random binding of the two substrates to the antibody. The rate enhancement may be due to overcoming entropy factors as a result of general binding and not to any specific interactions that facilitate the formation of new bonds. The initial reaction followed Michaelis-Menton kinetics; however, the reaction stopped after 10% completion -- perhaps due to strong product inhibition.

A *cis-trans* isomerization is an example of a reaction that involves both bond breakage and bond formation. The catalytic center must have a strong nucleophile or base to form a transient single bond, and moreover, must have sufficient flexibility to accommodate the rotation of the various moieties. The disubstituted piperidinium hapten in Figure 4 was used to elicit antibodies that catalyzed the isomerization of the *cis* isomer of an alpha-beta unsaturated carbonyl to the more stable *trans*.[5] In the minimum energy conformation of the hapten, the nitrophenyl moiety is close to the perpendicular position in the transition state that is needed for enone isomerization. Also, the positively charged amino group should induce a carboxylate moiety in the antibody binding site that may be capable of 1,4 nucleophilic addition to the enone. After preparation of the hybridomas, 15 monoclonal antibodies were isolated that bound the hapten. Of these, 3 catalyzed the isomerization (one produced a 15,000 fold rate enhancement) and demonstrated saturation kinetics (i.e. Michaelis-Menton) typical of an enzymatic reaction. The antibody was also inhibited competitively by the hapten.

FIGURE 1. CARBONATE HYDROLYSIS*



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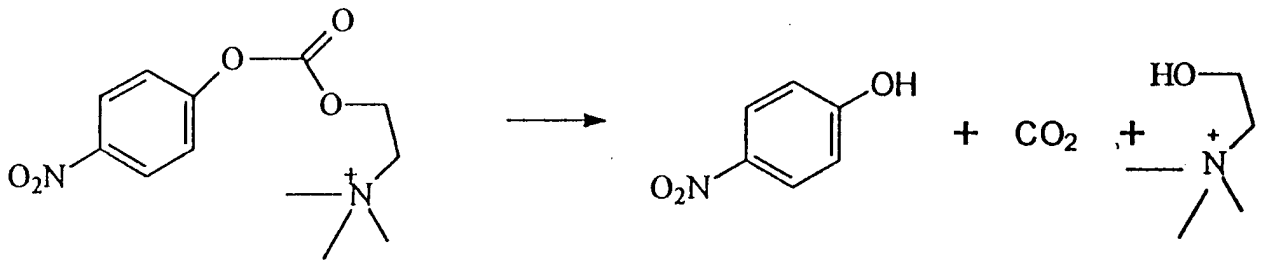

*POLLACK, JACOBS, and SCHULTZ, 1986

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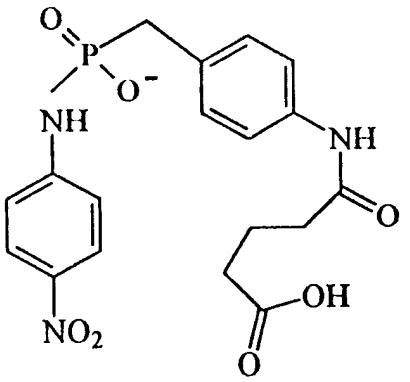
FIGURE 2. AMIDE HYDROLYSIS*



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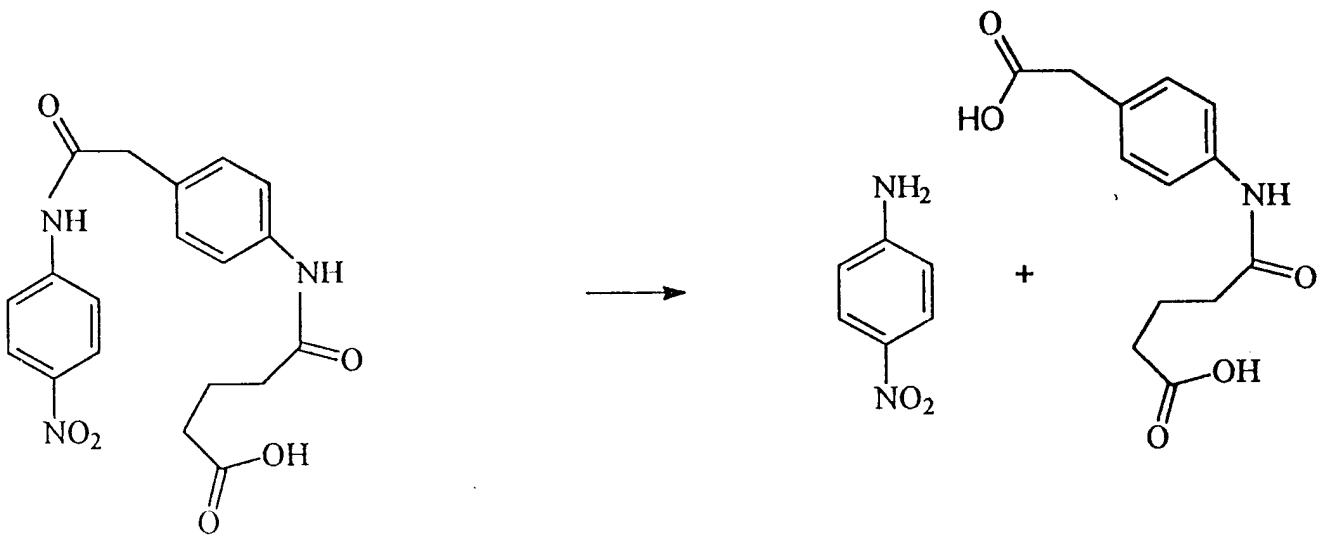
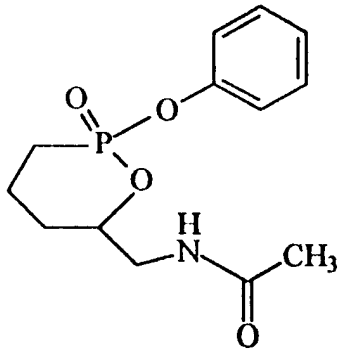


*JANDA, SCHLOEDER, BENKOVIC, and LERNER, 1988

FIGURE 3. AMIDE FORMATION*



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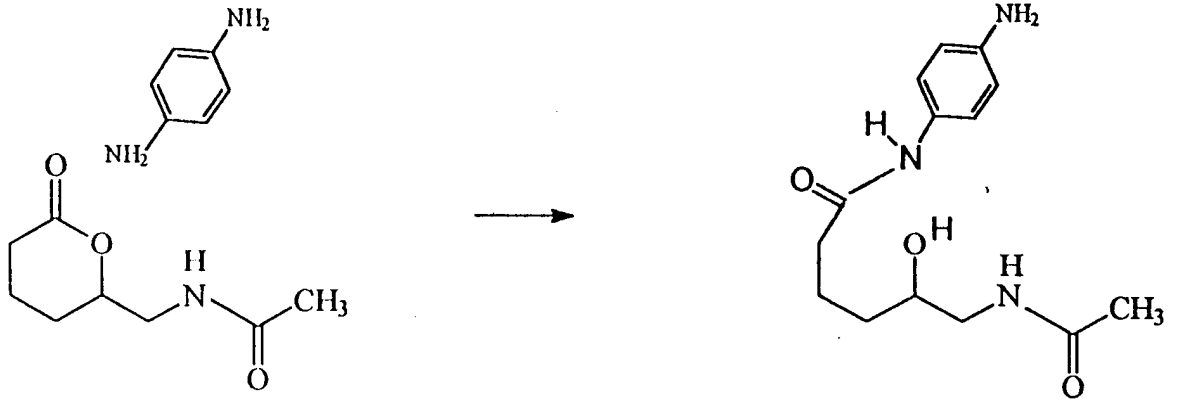


*BENKOVIC, NAPPER, and LERNER, 1988

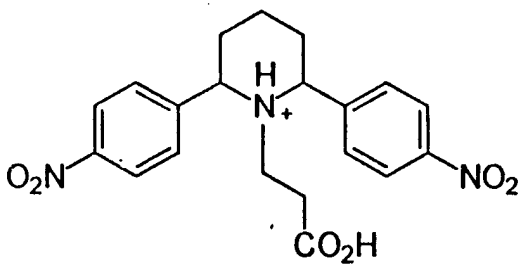
The Diels-Alder reaction is one of the most valuable synthetic tools because it generates two bonds simultaneously. The bimolecular (2 +4) cycloaddition has a very large entropy barrier due to the high degree of ordering in the transition state. Two separate molecules come together in a specific orientation so that the four reaction centers are positioned correctly for bond formation. Calculations indicated it might be possible to overcome the activation entropy resulting from loss of translational and rotational freedom by binding the two reactants to an antibody. Because the transition state occurs late in the reaction, it resembles the products more than the reactants. Unfortunately, if the product is used as the hapten, tight binding of the product to the antibody would prevent efficient turnover (as was seen in the example in Figure 3). In Figure 5, tetrachlorothiophene dioxide forms an unstable intermediate with N-ethyl maleimide that subsequently loses SO_2 to form dihydro-N-ethyltetrachlorophthalimide.[6] A stable analog of this intermediate served as a hapten for eliciting antibodies. The catalyzed reaction was first order with respect to antibody concentration. N-ethyl malimide was a good substrate whereas malimide was not thereby indicating the specificity that can be achieved in antibody catalyzed reactions.

Hydrogen abstraction reactions from carbon centers are used to generate olefins, isomerize certain molecules, and form a essential part of the aldol and Claisen condensations. In biological systems, proton abstractions are usually effected by glutamate or aspartate residues that have higher than normal dissociation constants (i.e. 10^{-6} to 10^{-8}). A positively charged hapten was used to generate a binding site containing a carboxylate residue.[7] Six monoclonal antibodies were elicited that bound the hapten illustrated in Figure 6. Of these, four catalyzed the beta-elimination reaction yielding both *cis* and *trans* isomers. The dependence of the reaction rate upon pH indicates that the catalysis is attributable to a single titratable group. The pKa for the active site is 6.2 and comparable to the value of 6.5 for the glutamate residue in the active site of carboxy-peptidase.

Although catalytic antibodies are useful in accelerating known reactions, their use in catalyzing unfavored reactions affords them a unique role in synthetic chemistry.[8] For example, the uncatalyzed epoxide opening illustrated in Figure 7 follows Baldwin's rules for cyclization reaction and gives the 5-exo product. Formation of the 6-endo product does not occur normally. Antibodies elicited to the N-oxide hapten catalyze the formation of the this unfavored product.[9] To achieve this phenomenon, the antibodies lowered the activation energy for the 6-endo product to a value less than that for the 5-exo product.

Ab initio studies at second order perturbation level using the 6-31G* basis set on the two epoxide opening reactions predicted that the activation energy for the 6-endo reaction would be 1.9 kcal/mole higher than for the 5-exo reaction.[10] This energy difference would yield a product ratio of 96:4 at 25 degrees centigrade. The calculations were consistent with experimental results because only a trace of the 6-endo isomer was observed. If the less favorable product is to be produced, the catalytic antibody must reduce the energy of activation for the 6-endo product at least 3.5 kcal/mole more than it reduces the 5-exo product. Reducing the activation energy only 1.9 kcal/mole would lead to the formation of equal concentrations of isomers. Comparing the calculated transition

FIGURE 4. CIS TRANS ISOMERIZATION*



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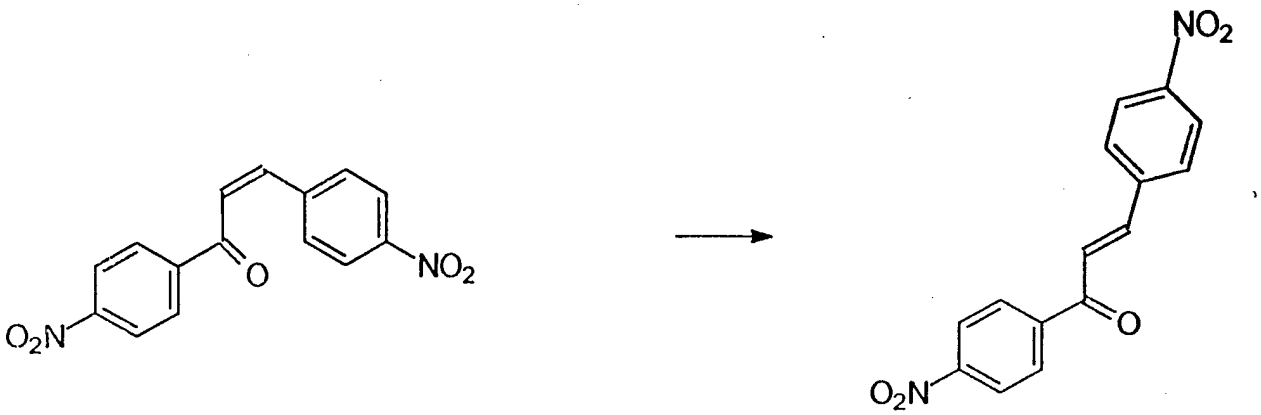
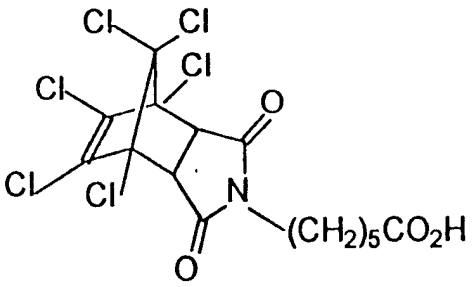


*JACKSON and SCHULTZ, 1991

FIGURE 5. DIELS ALDER REACTION*



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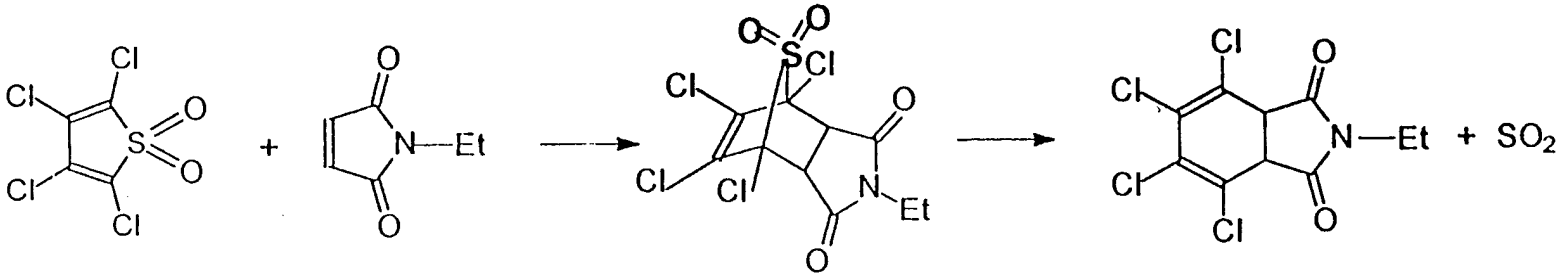


*HILVERT, NARAD, and AUDITOR, 1989

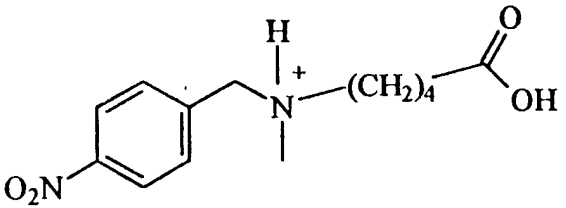
structures for the two reactions with the electronic features of the hapten helps understand how the antibody catalyzes the reaction. The positively charged carbon in the 6-endo transition structure is in a similar location to the positively charged nitrogen in the hapten. Thus the 6-endo transition structure is better stabilized by the antibody elicited to the hapten.

The previous example describes the use of computational chemistry to explain how a catalytic antibody shifts the reaction from one path to another. Can it be used to design a hapten to alter the reaction from one path to another? Mustard (*bis*-2-chloroethylsulfide) in aqueous solution undergoes an S_N1 type of reaction to generate a variety of electrophilic intermediates that react with proteins and other nucleophilic biomolecules. Any enzyme or other catalysts having a nucleophilic center would most likely be alkylated quickly by the mustard intermediate and subsequently undergo conformational changes leading to loss of activity. In contrast, proteins catalyzing an S_N2 type of reaction may be spared from alkylation. Computational chemistry at the semiempirical level (AM1 and PM3) indicated the transition structure for the S_N2 reaction was a distorted trigonal bipyrimid with the angle between the forming carbon-oxygen bond and the breaking carbon-chlorine bond about 145 degrees.[11] The bond length for the carbon-chlorine bond is about 2.4 angstroms. Considerable charge separation occurs in the transition structure with positive charge on the carbon atom and negative charge on the oxygen of the incoming water molecule and also on the departing chlorine ion. The calculated transition structure was use to design a stable analog that could be used to develop catalytic antibody binding sites. Stable trigonal bipyrimid compounds that are stable in physiological conditions and relatively non toxic are rare. Therefore, the decision was made to use a single methyl group to simulate the pair of hydrogens in the trigonal plane with the central carbon. Figure 8 compares the geometry and electronic structure of the proposed hapten with the transition structure. The Se-P-N bond angle is about 120 degrees and therefore considerably less than that for the transition structure. The overall size of the hapten is about the same as transition structure. The bond length of the P-Se bond is 2.3 angstroms and approximates 2.4 angstroms for the breaking carbon-chlorine bond. Synthetic efforts are underway to prepare the hapten and a protein conjugate for use in eliciting antibodies for hydrolyzing mustard in an organic medium.

SUMMARY

Catalytic antibodies bridge the gap between traditional chemistry and biology. Creative insight in designing and synthesizing stable simulants of transition structures and their subsequent application in inducing immunoglobulins have led to numerous monoclonal antibodies that accelerate the rate of naturally occurring reactions and allow the preparation of isomers not available by direct methods.

FIGURE 6. ELIMINATION REACTION*



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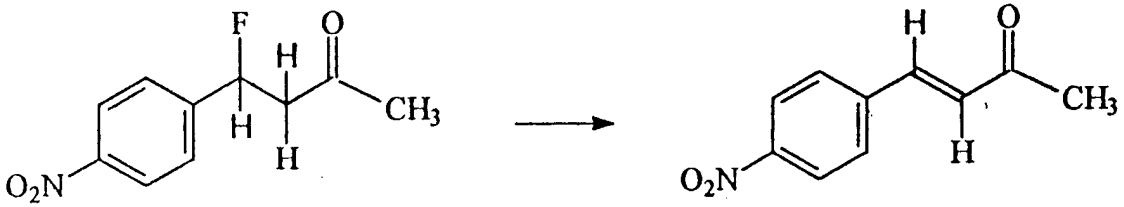
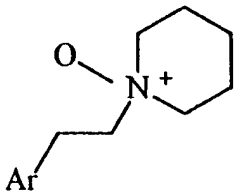


*SKOKAT, LEUMANN, SUGASAWARA, and SCHULTZ, 1989

FIGURE 7. PRODUCT SPECIFICITY*



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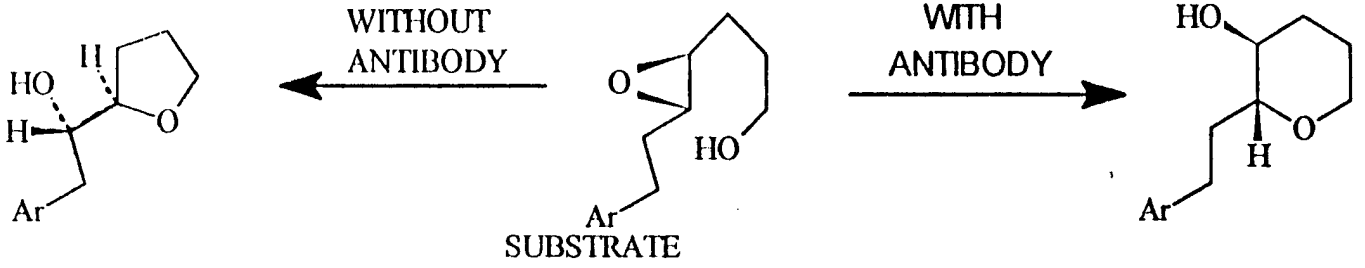
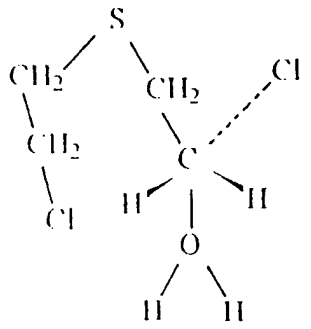


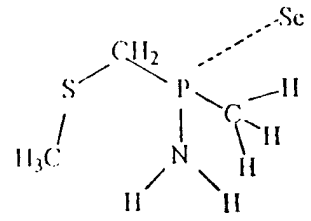
SUBSTRATE

*JANDA SHEVLIN, and LERNER, 1993

FIGURE 8. BOND LENGTHS AND ANGLES FOR MUSTARD TRANSITION STRUCTURE AND TRANSITION STATE ANALOG



BREAKING	FORMING	ADJACENT	ANGLE	Q(CEN)	Q(BRK)	Q(FM)
C--Cl	C--O	C--C	Cl-C-O	C	Cl	O
2.42	1.86	1.48	144.6	.15	-.71	-.33



P--Se	P--N	P--C	Se-C-N	P	Se	N
2.30	1.80	1.85	110.1	(2.0)	(-1.1)	(-.64)

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